

Radiation Usage Impact of on Anti-Nutritional Compounds (Antitrypsin and Phytic Acid) of Livestock and Poultry Foods

Mohammad Khosravi, Ali Kiani, Behroz Dastar, Parvin Showrang

Abstract—Review was carried out on important anti-nutritional compounds of livestock and poultry foods and the effect of radiation usage. Nowadays, with advancement in technology, different methods have been considered for the optimum usage of nutrients in livestock and poultry foods. Steaming, extruding, pelleting, and the use of chemicals are the most common and popular methods in food processing. Use of radiation in food processing researches in the livestock and poultry industry is currently highly regarded. Ionizing (electrons, gamma) and non-ionizing beams (microwave and infrared) are the most useable rays in animal food processing. In recent researches, these beams have been used to remove and reduce the anti-nutritional factors and microbial contamination and improve the digestibility of nutrients in poultry and livestock food. The evidence presented will help researchers to recognize techniques of relevance to them. Simplification of some of these techniques, especially in developing countries, must be addressed so that they can be used more widely.

Keywords—Antitrypsin, gamma anti-nutritional components, phytic acid, radiation.

I. INTRODUCTION

SECURITY and food are the most important global issues today, since more than 800 million people suffer from malnutrition, while many women and children face the pain of hunger [1]. In this regard, proteins are the most worrying. Grains, as the main sources of protein, are the supplementary of cereals in the balance of amino acids. On average, grains and cereals contain 20-30% protein and 14 MJ metabolizable energy. They also include calcium, magnesium, iron, zinc, potassium. As protein sources, grains are cheaper than animal protein sources [2]. Grains and cereals (corn, soybeans, wheat, barley, canola and sorghum, etc.) are the most dominant elements in animal foods. Grains and cereals contain anti-nutritional compounds including anti-trypsin, phytic acid, alpha-amylase inhibitors, oligosaccharides, lectins,

Mohammad Khosravi is with Department of Animal and Poultry Nutrition, Faculty of Animal Science, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran (phone: +98 911 438 3082; e-mail: mohammad.khosravi121@gmail.com).

Ali Kiani Author is with the Livestock production systems Institute of George-August university, Gottingen, Germany (corresponding author to provide phone: 0049-17629490514; e-mail: alikianee@gmail.com).

BehrozDastar is with Department of Animal and Poultry Nutrition, Faculty of Animal Science, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran (phone: +98 911 371 6862; e-mail: Dastar392@yho.com).

ParvinShowrang is with Radiation Applications Research School, Nuclear Science and Technology Research Institute, Atomic Energy Organization of Iran, Karaj, Iran (e-mail: pshawrang@nrcam.org).

glucosinolates, cyanogenic, allergens, saponins, alkaloids, etc. [3], [4]. Some processing methods such as steaming [5], extruding [6], use of germination chemicals [7], fermentation, using of extraction solution with the acid and alkaline compounds and enzymes have been used for food processing [3], [7], [8], but none of mentioned methods are able to eliminate these compounds completely. Nonetheless, using the combination of food processing methods is better. The advantages and disadvantages of the mentioned methods were recently extensively discussed, but among them, food irradiation is mainly considered. Radiation which is known as a physical processing method includes controlled use of the energy of ionizing rays such as electron and gamma rays to improve the value of foodstuffs. Using ionizing radiation causes less damage to the nutrients, especially protein, fewer indigestible reactions such as the Maillard reaction, a reduction of microbial and fungal contamination, as well as a decrease in the anti-nutritional factors and an increase the digestibility of nutrient [4]. The aim of this paper is to review the effects of radiation on anti-nutritional characteristics (antitrypsin and phytic acid) and biological value foodstuffs. This paper aims to present information to motivate the further research in this area and also to promote the plant as protein and carbohydrates sources in nutrition.

A. Using Radiation

There are various methods to deactivate or reduce anti-nutritional substances such as heating, boiling, soaking in water, using alcohol and acid, budding, and fermentation [7]-[10]. All the mentioned methods not only can eliminate all the anti-nutritional ingredients but also have undesirable impact on the characteristics of the final product. In recent decades, the irradiation process is one of the common food processing methods, which causes less destruction to nutrients, especially proteins, does not create indigestible products like Millard products (creation of non-hydrolysable link between carbohydrates and proteins due to heating), and results in the removal of microbial and fungal contaminates from the nutrients, elimination of anti-nutritional foods and increased nutrient digestibility [4]. Gamma and electron radiations are ionized beams with enough energy to ionize atoms. Electron and gamma rays ionize atoms through the transfer of energy, directly and indirectly, to the electrons. The chemical effect of electron irradiation is quite similar to gamma irradiation [11].

B. Irradiation Processing

When a radioactive atom decays, it produces one or more types of ionizing ray. These rays have enough energy to ionize the atoms, which are in the electrons or irradiated particles, to form the nucleus (alpha and beta particles) or electromagnetic ray from nuclear (gamma ray) or from the electric field around the core (X-ray). When the ratio of neutrons to protons in a radioactive isotope is very low, alpha particles emit from the nucleus, due to the two load positive charges (++) and low speed in alpha particles that have high ionizing power but little penetration (up to a few centimeters in air and a few millimeters in tissue) [8], while the beta particle is a common electron imitated from an unstable nucleus after processing a neutron to a proton. Beta particles have more ionizing power than alpha particles, but greater leverage (up to several meters in the air and a few millimeters in tissue). When a neutron changes to a proton, the nucleus, which still has more energy, excretes a gamma photon to reach greater stability. Thus, often a gamma ray emits following a released beta particle. Ionizing the atoms has been done through alpha, beta rays, and electrons directly, but the X- and gamma rays ionize the atoms by transferring their energy to electrons indirectly. Gamma rays have no mass and electric charge, then are not influenced by magnetic and electric fields and move directly with light speed and high penetration power [11]. Due to its very short wavelength (0/03 to 0/003 nm), gamma rays are the most penetrable and energetic photons in the electromagnetic spectrum. As soon as gamma-rays hit an atom, its energy is transferred to the electron on the orbital then causes ionization of atoms snatching a high-energy electron [12]. In this case, gamma rays disappear or its decreased energy transfers to the electron. These effects may alter chemical bonds directly or indirectly and cause changes in some molecule structure.

C. Quantity of Ionizing Radiation Units

A unit of energy of ionizing rays is usually an electron Volt (eV), which is the amount of kinetic energy gained (or lost) by the charge of a single electron moving across an electrical potential difference of one volt in vacuum. 1eV is 1.6×10^{-19} Joule (J). The dose quantity is used to determine the amount of energy transferred to the material through the ionizing radiation in the unit of Gray (Gy). Gray is defined as the absorption of 1 joule of radiation energy per kilogram of matter [4].

D. Source of Ionizing Radiation

Radionuclides, as radioactive materials with ability of ionizing radiation, are the main sources of ionizing rays. Gamma rays emitted from radionuclide have enough penetration ability into the food and meet all the needs of irradiation, without generating any radioactive by-products. Cobalt-60 or Caesium-137 with 1.25 and 0.66 MeV energy, respectively, are the approved radionuclides for food irradiation. By emitting alpha particles and gamma radiation, Cobalt-60 changes to non-radioactive nickel [13]. An electron ray is generated by an accelerator engine with high electric power (10 Million eV energy). The electron accelerator is an

electrical system made up of two main parts; 1) electron source and 2) an accelerator electric field. High-energy accelerated electrons from an electron gun can be driven in a straight line, and no radioactive source is required for the accelerated electron. Electrons are able to penetrate into foods by as much as 5-10 cm, and their generation stops by cutting off the electric flow. Due to their short wavelength and high permeability, gamma and X-rays can penetrate into materials of more than 300 mm depth. Gamma and X-rays radiation ability and processing speed is less than the electron radiation, consequently makes them suitable for the processing of a wide range of products, while the X- and gamma rays have been employed for sterilizing medical instruments and food protection [14]. Because of more complications with respect to gamma radiation systems, electron radiation is just used in small diameter and in small packing. The only main factor in determining the absorbed dose in radiation product is time of radiation. Technological complexity of gamma irradiation systems than the other common types of radiation to measure the time of radiation is easier and simpler. Hence, the gamma radiation systems are most commonly used [14].

E. Calibration and Dosimetry of Radiation System

Successful radiation with defined doses and desirable results needs a level of knowledge of the amount of output energy per time unit (rate of radiation), the distance between the source of radiation and the target, material exposed to radiation for a defined time, accurate measurement of the absorbed dose in the target material, as well as determination of the distribution dose throughout the material and control the process from the beginning up to the end. Considering that the energy resources (gamma, electron beam or X-ray) lose their energy over time, working with any system needs appropriate calibration and radiation dosimetry based on the standard procedure [15].

The measurement of an absorbed dose is possible using an appropriate dosimetry system. Also, absorbed dose is similar to the temperature in crescent that reflects the same concept of absorbed energy per mass unit.

An absorbed dose of 10 kGy is equivalent to 2.4 calories per gram, which increases the water temperature up to 2.4 °C. Because the specific heat of water is quite large, it can contain a relatively large amount of thermal energy per unit mass with a relatively small increase in temperature. Since the liquid water is relatively easy to transport from one location to another, large quantities of energy can be moved from one place to another with relative simplicity by water.

Another dosimetry depends on the chemical effects of the radiation, while the other practical dosimetry systems are available in various forms of liquids, solids, and films. Thin film contains colors sensitive to radiation, which has been applied in electron beam irradiation system [1].

F. The Use of Radiation to Improve the Nutritional Value of Livestock and Poultry Foods

Many researches have been conducted on the use of electron and gamma ray irradiation to improve digestibility or

to change the digestion and absorption location of nutrients and remove the anti-nutritional factors of foods. The results of these studies have shown that more than 10 kGy radiation has the effect on deactivation of anti-nutritional compounds such as; tannins, gossypol, protease inhibitors, lectin, phytic acid, non-starch polysaccharides, and oligosaccharides without any change in the nutritional quality of the food [4]. Higher levels of 50 kGy and 250 kGy (Mac Manus and Manta, 1972) in order to improve the degradability of crude protein and NDF of forage and outflow of grain are used, respectively [16]-[18].

G. Phytic Acid (Hexa-Inositol Phosphate)

Phytic acid (*myo*-inositol 1, 2, 3, 4, 5, 6 Hexandihydrogenphosphate) is stored in the form of phosphorus in plant seeds, so that 65% to 70% of phosphorus of plant sources is in the phytate phosphorus form [19]. Grains and cereals which are normally used in poultry diets contain similar amounts of phytic acid (approximately 25% dry matter). Phytic acid combines minerals to form in phytin complex plants. Then, phytin can join to protein vacuoles within the membranous protein [20], [21].

Lack of proper enzymes to hydrolysis phytates to inorganic phosphate and inositol, causes some limitations in phytate usage in some monogastric animals including poultry. Since phytic acid reduces the availability of minerals, monogastric animals are limited in the use of phytate because of lack of phytase enzyme [21]. In general, there is a negative correlation between the amount of contained phytate in diets and the digestibility of multivalent cations. In neutral pH, phytate can combine to polyvalent cations to form insoluble complexes that are resistant to the process of digestion and absorption in the gastrointestinal tract; as a result, part of minerals which form daily diet will be inaccessible to birds [22]-[24]. Some enzymes such as alpha-amylase, calcium, and others (phosphatase, carboxypeptidase, and aminopeptidase) contain zinc. By increasing the amount of phytate in the diet, the activity of these enzymes reduces and can have a negative effect on the use of protein and starch. The role of calcium and zinc in insulin secretion is very important. Calcium deficiency significantly reduces the secretion of insulin. Zinc is also involved in the transfer and storage of insulin. On the other hand, conversion of proinsulin to insulin requires the presence of large amounts of trypsin. The presence of phytate in the diet reduces the activity of trypsin [25]. Phytate, with six hydroxyl groups in its chemical protein structure, also has the ability to create an ionic bond. Ionic bonding leads to a decrease in protein solubility, therefore reduces the usability of proteins and amino acids. Phytic acid can be combined to digest enzymes, such as trypsin and pepsin in the gastrointestinal tract and reduces their activity [24]-[30].

H. Ionizing Rays Effect on Phytic Acid

A reduction in phytic acid causes a linear increase in radiation dose [31]. Reference [32] reported that gamma rays reduce the amount of phytic acid in soybeans. Also, [23], [24] demonstrate that a reduction in concentrations of phytic acid in irradiated raw and cooked vetch occurred with 2, 4, 6, and

10 kGy doses of gamma ray. Concentration of phytic acid in cottonseed meal with a 30 kGy dose of gamma and electron radiation reduces up to 85% and 74%, respectively [33]. In another study, the researchers reported that electron radiation to canola meal for a 15 kGy dose reduces phytic acid over 90%, while 30 kGy and 45 kGy doses result in the complete disappearance of phytic acid [34]. Reference [35] reported that gamma ray doses of 15 kGy to 30 kGy completely destroy phytic acid.

Reference [36] reported that soybeans treated with a dose of 15 kGy gamma ray, reduced phytic acid by 59% compared to a control treatment. They also showed that 30 kGy and 45 kGy doses of gamma radiate can completely eliminate the phytic acid content of soybeans. The effects of 15 kGy, 30 kGy, and 45 kGy doses of electron radiation on the content phytic acid of soybeans were studied, and the results show that at 15 kGy, the doses of electron reduce the amount of phytic acid at a rate of 43% compared to the control, while doses of 30 kGy and 45 kGy of electron, completely removed the anti-nutrient factor from soybeans [37]. A study of the effect of 15 kGy, 30 kGy, and 45 kGy doses of electron beam on the amount of phytic acid in canola seed has shown that a 15 kGy dose reduces the content of phytic acid by 83% compared to the control, while 30 kGy and 45 kGy doses of electron beam completely removed this anti-nutrient agent in canola seeds [23], [24].

Another study on the effect of cotton seeds treated by an electron beam on phytic acid demonstrated that, for 15 kGy doses of an electron beam, the amount of phytic acid decreased by 58% compared to the control, while 30 kGy and 45 kGy doses completely eliminated the phytic acid content in cotton seeds [37]. Decrease in amount of phytic acid caused by radiation, is probably because of photolysis phytate to inositol and inositol phosphate by the activity of free radicals produced by radiation, or by phytate ring.

I. Trypsin Inhibitor

Serine protease inhibitors (Serpins) consist of 400 amino acids which contain 3 beta sheets, 7-9 alpha helix, and also a reactive loop. Alpha-1- anti-trypsin serine protease inhibitor, as a polymorphic glycoprotein, is a member of the 52KD family which is coded mainly by a single gene on the long arm of chromosome 14 [38], [39].

The main role of Hepatocytes and monocytes is protease inhibition. Degradation in a reactive loop of serpin has been done after anchoring this loop in an active site of protease. This reactive loop has been broken by protease, while protease attached to it quickly goes inside of beta sheet by mouse trap mechanism and causes changes the conformation of the protease [40]. Derived serpin-protease complex is quickly removed by blood flow through multiple receptors, including enzyme-serpin complex receptors, serpin receptors, LDL, and VLDL receptors. Trypsin inhibitor, as anti-nutrient elements in rice bran, can decrease the performance (e.g. body weight gain and feed conversion ratio) of broiler chickens [41]. This inhibitor causes hypertrophy in the pancreas and also increases the relative weight of pancreas to the whole-body trypsin inhibitors. It causes abnormal development in ratio of pancreas

weight to the total body, abnormal profile of amino acids and exclusion of trypsinogen enzymes which contain cysteine. Exclusion of these enzymes results in an imbalance of consumed amino acids, which leads to disruption in metabolism and decrease the performance [25].

J. Effect of the Radiation Beam on Trypsin Inhibitor

Soybean which is exposed to gamma of 10 kGy dose radiation decreased to 54.5% in activity of trypsin inhibitor. In another study researches reported that inactivation of trypsin inhibitor linearly increases by increased irradiation dose. This means that radiation in 5, 15, 30, and 60 kGy, reduces the activity of trypsin inhibitor to the 41/8, 56/3, 62/7 and 72/5 percent, respectively [42]. Reference [15] demonstrated that in 1, 2, 4 and 5 kGy dose gamma emitters, causes 5.6, 10.4, 38, and 45.1% reduction in trypsin inhibitor respectively in dry Beans. Effect of gamma rays on trypsin inhibitor activity in safflower showed that trypsin inhibitor in 42 kGy doses has been completely disabled [43]. Reference [44] reported in 100 kGy dose gamma emitters, the trypsin inhibitor activity in soybean decreased to 98%. Soybean trypsin inhibitor activity in irradiated soybean by 15, 30, and 45 kGy dose gamma ray declines by 18.4, 55.5, and 63.5% respectively, compared to the control treatment [36]. Reference [45] observed that activity of trypsin inhibitors in irradiated soy flour decreases to 34.9% with 10 kGy gamma radiation dose. In another study that was conducted by [37], 19, 73, and 88% reduction in trypsin inhibitor activity has been observed in 15, 30, and 45 kGy doses of electron beams, respectively. In another study, researchers found that the amount of trypsin inhibitor in broad beans by 2.5, 5, 7.5, and 10 kGy doses in gamma ray declined 4.5, 6.7, 8.5, and 9.2%, respectively [46]. Trypsin inhibitor can be inactivated by destroying the disulphide bonds and sulfhydryl groups [4], [47]. Sulfur-containing aromatic acids are more sensitive to radiation. The reaction of hydroxyl and superoxide anionic radicals generated during gamma irradiation with amino acid of polypeptide chain, causing amino-radicals acid and followed by cross-linking the radical-amino acids of polypeptide of different chains. Therefore, gamma radiation can change the structure of protein and molecular characteristics by disjoining and generating cross-linking, and then, loss of biological characteristics of proteins such as trypsin inhibitor enzyme occurs. Trypsin inhibitors deactivation by gamma irradiation is due to degradation the sulfide groups through the ionizing beam. It seems that degradation of trypsin inhibitor enzyme by irradiation leads to decrease in amount of this enzyme [36].

K. Improvement of Degradability

Cross-linking and protein linkage through ionizing beams, create resistant linkage to digestion which reduces accessibility of microbial enzymatic microbes to substrate, then results in increase in rumen bypass protein from the small intestines. Different studies demonstrate that more than 35 kGy doses bind proteins with high molecular weight [12], [48], [49].

Soybean meal treated with higher kGy doses of gamma

radiation has less degradation of crude protein in rumen. In all cases, successful use of protected protein in the rumen against degradation needs to consider their digestibility in intestine. In another study, they reported that increase in the dose of gamma radiation causes not only reducing protein degradation of rapeseed meal in the rumen but also increases intestinal protein digestibility [16], [17]. The greatest impact on increasing the digestibility treated with gamma radiation was in dose of 75 kGy. In crescent of hydrophobicity in the surface of protein due to separating hydrogen bonds and weak non-covalent links and change the amino acid side groups through radiation concludes intestinal digestibility of crud protein of meals. According to the side group of hydrophobic amino acids which are the active chemical grope of pepsin, trypsin, and chymotrypsin enzymes, this processing procedure produces a suitable situation for intestinal trypsin and chymotrypsin enzyme activity [50]. Gamma radiation and electron beams are involved in improvement the bioavailability of carbohydrates in lignocellulosic compounds due to breaking the lignin-carbohydrate bonds [4]. Gamma irradiation (200-50 kGy) can be used to reduce cell-wall components of lignocellulosic materials in agricultural residues [51]. Treatment with 50 kGy dose of gamma radiation has no effect on the amount of NDF and ADF. Above 50 kGy dose, gamma radiation leads an increscent to degradability of dry matter in rumen [52]. Combination of radiation and sodium hydroxide usage shows better effect.

Using alkaline after radiation process reduces the amount of cellulose and hemicellulose, due to their degradation into the soluble matters. Penetration of sodium hydroxide solution into the complex lingocellulose synchronous with radiation on lingocellulose construction increases the reaction rate [53].

Higher dose of 100 kGy electron beam can cause depolymerization of cell-wall components such as cellulose. Therefore, insoluble cellulose treated by radiation dissolves and changes into rapid degradation phase. Electron irradiation by producing free ions and radicals causes depolymerization of complex compounds, especially separation of hydrogen bonds in cellulose, breaking the linkage between the cellulose and other compounds and breaking the covalent bonds [52], [54]-[56]. To determine the of gamma radiation effects on chemical composition and degradation of dry matter of alfalfa hay, researches used 75, 50, and 25 kGy gamma radiation doses and reported that more than 50 kGy increases degradation of dry matter of dry alfalfa in the rumen [57]. In [58], the effects of the electron beam on chemical composition and cell-wall degradation in the rumen of cow; 100, 200, and 300 kGy doses of electron beam to processing wheat straw and barley straw have been studied using by rodotron machine. This processing treatment significantly decreased the amount of NDF and ADF. Decreasing in cell-wall of wheat straw by electron beam irradiation is because of dissolving of cellulose and more hemicellulose (by the releasing from cellulose).

L. Increscent of Digestibility

In most non-manipulated food proteins, hydrophobic amino

acids are in inner core of the protein, and can be exposed due to radiation and protein denaturing. Aromatic amino acid are chemical reactive groups of pepsin enzyme, hence high amount of aromatic and hydrophobic groups on the surface of protein through the radiation causes increase in digestibility [16], [17]. Denaturing is the main reason of more digestibility of crude and true protein in irradiated seeds.

Denaturing leads to replacement of amino acids position with hydrophobic side groups from nucleus to the surfaces of molecules and causes more hydrophilic property in proteins surface which concluded more digestibility in protein [59]. Enhancement in dry matter digestibility of irradiated grain is due to the elimination of enzyme inhibitors [4]. As reported by [60], [61], some researchers have shown that gamma radiation increases the nutritional value of rye and barley in feeding broilers.

Increase in nutritional value is probably because of dipolymerization of soluble beta-glucan and pentosans. Some problems with adhesion excretion and rectal infections were observed in fed chickens with untreated rye and barley because of their anti-nutritional compounds. In fed chickens with irradiated seeds, all these problems have been omitted due to reduction or elimination of these anti-nutritional compounds. In addition, the digestibility of fat, amino acids, and starch of irradiated seeds were higher, and performance of chickens was better than the control group [54], [60], [61].

M. Using the Radiation to Reduce the Microbial Contamination of Food

One of the problems in the poultry industry is food contamination with *Aspergillus fungi* and *bacteria* (*Salmonella* and *E. coli*). Contaminated food intake is too harmful for poultry and causes reduction in conversion rate, reducing production efficiency, increasing the mortality, and at least increasing economic costs of breeding. Moreover, some of these contaminations can transmit to human through poultry products. Several physical and chemical methods have been used to reduce fungal and bacterial contamination. Chemical methods included the use of phenol and phenol derivatives, quaternary ammonium compounds, iodine compounds, chlorine products, alcohols, organic acids, and the physical means consist of heating and radiation [12], [62]-[65]. Phenols are effective on the fungi, gram-positive, gram-negative, and some viruses but have no impact on bacterial spores. Quaternary ammonium compounds are effective against gram-positive bacteria, but have relative effect on gram-negative bacteria. Chlorine as a caustic matter can be activated by organic materials rapidly, and quickly loses its effectiveness. Iodine compounds are effective against gram-negative and gram-positive bacteria, but have little effect on bacterial spores. Using the organic acids not only prevents the growth of harmful microorganisms in ingredients of stored foods but also inhibition the pathogenic microorganisms, such as *Salmonella* and *Escherichia coli* in the digestive tract. Organic acids are corrosive for equipment and even are one of the environmental threats [66]. Heating by microwave and irradiation are some methods to protect food from

contamination [67]. Based on time exposing to the sunlight, aflatoxin can be omitted [68]. Researchers report that being 10 minutes under sun rays reduces content aflatoxin of peanut oil to 75%. Time and penetration of sunlight are important factors in this process [69].

However, radiation by microwave, such as the other heating methods, can destroy pathogenic microorganisms, heating the contaminated foods by microwave for 2, 4, 8 and 10 minutes had least impact on degradation of aflatoxin. These results imply that most of the mycotoxins are resistant to heat. Gamma rays and electrons have a significant role in eliminating of fungal, toxin, and some bacterial contamination of food [70]. By the 15, 20, and 30 kGy doses of gamma radiation, aflatoxin B1 can be reduced by 55 to 64% [71].

Reference [68] found that gamma ray is significantly effective on aflatoxin concentration of food as increasing in irradiation dose causes increase in aflatoxin disintegration. In [72], [73], it was reported that 20 kGy dose of gamma radiation reduced aflatoxin by 83 to 100%. 10 kGy dose of gamma radiation is enough to complete disintegration of aflatoxin B1 contamination in corn [74]. Reference [75] reported that maximum clearance of infected samples in 10 kGy dose is just 80%. The difference between these two studies may be related to the amount of aflatoxin contamination. As a result of the previous study, degradation of aflatoxin B1 in corn, pistachios and peanuts, based on the oil content, by 16, 32 and 38 oil content is 80, 69, and 58%, respectively. Bacteria are less resistant to radiation, and the 3-7 kGy dose is sufficient to remove them [75].

II. CONCLUSION

Providing secure food to global population is a challenging task. Animal products and by-products foods play a vital role in addressing issue of food and nutritional security. Radiation usage is important tool in animal production research. These techniques united to the use of molecular tools are making possibilities to revolutionize the understanding of complex biological processes and make the livestock an efficient entity, highly productive with minimum inefficient expulsions to the environment, helping to achieve sustainability of the global food chain. Results of several studies showed that irradiation can remove the phytic acid and trypsin inhibitor. Gamma and electron irradiation through improvement the digestibility and absorption of nutrients can improve food conversion and weight gain in poultry and livestock.

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